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Polymorphisms among chloroplast and mitochondrial genomes of *Citrullus* species and subspecies

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Abstract

Twelve and six DNA clones representing various parts of chloroplast and mitochondrial genomes, respectively, were used to detect polymorphism among five watermelon cultivars and 21 U.S. Plant Introductions (PIs) collected from diverse geographical locations and representing major groups of *Citrullus* species. Cluster analysis based on 20 chloroplast DNA (cpDNA) and 10 mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) markers differentiated the accessions into three major phenetic groups: PIs and watermelon cultivars of *Citrullus lanatus* subsp. *vulgaris* (Schrad. ex Eckl. et Zeyh.) Fursa (also designated as C. *lanatus* var. *lanatus*) (group I), PIs of *C. lanatus* var. *citroides* (of *C. lanatus* subsp. *lanatus* Schrad. ex Eckl. et Zeyh.)(group II), and *C. colocynthis* (L.) Schrad. PIs (group III). The chloroplast and mitochondrial genomes of watermelon cultivars are distinct, but closely related to those of the *C. lanatus* var. *lanatus* PIs. On the other hand, the chloroplast and mitochondrial genomes of the wild species *C. colocynthis* are more similar to those of *C. lanatus* var. *citroides*. Polymorphic cpDNA and mtDNA markers identified in this study can complement isozyme and nuclear DNA data used in earlier phylogenetic and phenetic classifications of *Citrullus* PIs. These cpDNA and mtDNA markers are being used in experiments designed to enhance watermelon cultivars by replacing the chloroplast and mitochondrial genome of cultivated watermelon with those of the wild species *C. colocynthis*.

Introduction

The xerophytic genus *Citrullus* Schrad. ex Eckl. et Zeyh. thrives in the Old World tropics (Singh 1990) and comprises four known diploid ($n = 11$) species: *C. colocynthis* (L.) Schrad., *C. lanatus* (Thunb.) Matsum et Nakai, *C. ecirrhosus* Cogn. and *C. rehmii* De Winter. *C. colocynthis* (L.) Schrad. is a perennial bitter gourd which grows in sandy areas throughout northern Africa, Southwestern Asia, and the Mediterranean (Zamir et al. 1984; Burkill 1985; Navot and Zamir 1987; Jarret et al. 1997). *C. lanatus* (Thunb.) Matsum et Nakai is naturally grown in tropical and subtropical climates throughout the world but is considered to be native only in the dry sandy areas of Southern Africa (Bates and Robinson 1995). *C. lanatus* includes the following three subspecies: C. lanatus subsp. *lanatus* (Schrad. ex Eckl. et Zeyh.), *C. lanatus* subsp. *vulgaris* (Schrad. ex Eckl. et Zeyh.) Fursa, and *C. lanatus* subsp. *mucosospermus* Fursa. *C. lanatus* subsp. *lanatus* thrives in the Kalahari Desert where it is used as an essential source of water and food, and is named there 'tsamma'

melon. The 'citron' melon (also known as preserving melon; *C. lanatus* var. *citroides* [L.H. Bailey]) is a group of ancient cultigens in Southern Africa derived from the 'tsamma' melon. On the other hand, *C. lanatus* subsp. *vulgaris* is the desert watermelon group from which the red sweet cultivated watermelon is derived (Jeffrey 2001). In recent years, the red sweet cultivated watermelon has been designated as *C. lanatus* var. *lanatus* (Whitaker and Davis 1962; Whitaker and Bemis 1976; Burkill 1985; Jarret et al. 1997; USDA, ARS, Germplasm Resources Information Network / www.ars-grin.gov). *C. lanatus* subsp. *mucospermus* includes the 'egusi' melon cultivated in West Africa, primarily for the consumption of its oil and protein rich seeds (Jeffrey 2001). *C. ecirrhosus* Cogn. (Meeuse 1962) and *C. rehmii* De Winter (De Winter 1990; Singh 1990; Bates and Robinson 1995) are native to the desert regions of Namibia (Meeuse 1962; Jarret et al. 1997). The history of cultivated watermelon has not been sufficiently investigated (Jeffrey 2001), and taxonomic classification of various *Citrullus* types collected in the wild has yet to be validated, as indicated for *C. rehmii* De Winter (Robinson and Decker-Walters 1997; Jarret and Newman 2000).

During the last century, watermelon consumption has increased steadily, and today watermelon accounts for 2% of the world area devoted to vegetable production (FAO 1995). Genetic studies (Navot and Zamir 1987; Levi et al. 2001a,b) indicate that although the wide phenotypic diversity, there is a narrow genetic base among watermelon cultivars. Enhancing disease and pest resistance of watermelon cultivars and improving their response to environmental stress could be accomplished by increasing genetic diversity through hybridization with diverse types of *Citrullus* accessions. Phenetic relationships among the main *Citrullus* species and subspecies were examined by using isozymes (Zamir et al. 1984, Navot and Zamir 1987) and nuclear DNA markers (Jarret et al. 1997; Levi et al. 2001a). However, these studies did not clearly specify whether *C. colocynthis* is more similar to *C. lanatus* subsp. *lanatus* or to *C. lanatus* subsp. *vulgaris*. Also in these studies, a few of the PIs that are designated as *C. lanatus* var. *lanatus* (Germplasm Resources Information Network; GRIN /www.ars-grin.gov) were more similar in their DNA pattern to the group of *C. lanatus* var. *citroides*, (Levi et al. 2001a). Chloroplast and mitochondrial genomes may help determine phenetic relations among *Citrullus* accessions.

The objectives of this study were (1) to detect polymorphisms among chloroplast and mitochondrial genomes of *Citrullus* accessions collected from various geographical locations, (2) to determine phenetic relationships among *Citrullus* accessions based on organellar DNA polymorphisms and compare them with the phenetic relations based on nuclear DNA markers (Jarret et al. 1997; Levi et al. 2001a), and (3) to identify chloroplast and mitochondrial DNA markers that could readily differentiate between chloroplast and mitochondrial genomes of the major *Citrullus* groups.

Material and methods

Plant material

Seeds of PIs (Table 1) were obtained from the USDA, ARS, Plant Genetic Resources Conservation Unit (Griffin, Georgia). Seeds of the cultivars 'Allsweet', 'Black Diamond', 'Charleston Gray', 'Dixie-Queen', and 'New Hampshire Midget' (Table 1) were kindly provided by Syngenta Seeds (Napels, Florida). Seeds were germinated in the greenhouse, and 10 g of young leaves were collected from five plants (3 weeks old) of each PI or cultivar and stored at −80 °C.

Isolation of total DNA

In order to avoid co-isolation of polysaccharides, polyphenols, and other secondary compounds that damage DNA, a modified extraction procedure was used with high concentrations of CTAB (2.5%) and SDS (0.5%) in the DNA extraction buffer (Levi and Thomas 1999). Quality and quantity of each DNA sample were determined by using spectrophotometry and electrophoresis through a 1% agarose gel following Sambrook et al. (1989).

RFLP procedure for organellar genomes

DNA samples (1 μg each) were digested (at 37 °C for 12 h) with one of the following restriction

Table 1. Watermelon cultivars and U.S. Plant introduction accessions (PIs) used in the present study, the taxon (as designated in GRIN) and phenetic group (as classified in Figure 3; based on cpDNA and mtDNA markers in Table 2) to which they belong, and the country where they were collected.

Accession	Taxon	Group	Country
Allsweet	*C. lanatus* var. *lanatus*	I	USA (1972)
Black Diamond	*C. lanatus* var. *lanatus*	I	USA (1945)
Charleston Gray	*C. lanatus* var. *lanatus*	I	USA (1954)
Dixie Queen	*C. lanatus* var. *lanatus*	I	USA (1890)
New Hampshire Midegt	*C. lanatus* var. *lanatus*	I	USA (1951)
PI 162667	*C. lanatus* var. *lanatus*	I	Argentina
PI 169289	*C. lanatus* var. *lanatus*	I	Turkey
PI 185635	*C. lanatus* var. *lanatus*	I	Ghana
PI 189317	*C. lanatus* var. *lanatus*	I	Zaire
PI 270306[a]	*C. lanatus* var. *lanatus*	II	Philippines
PI 179881[b]	*C. lanatus* var. *citroides*	II	India
PI 189225	*C. lanatus* var. *citroides*	II	Zaire
PI 244019	*C. lanatus* var. *citroides*	II	S. Africa
PI 271778[c]	*C. lanatus* var. *lanatus*	II	S. Africa
PI 296341	*C. lanatus* var. *citroides*	II	S. Africa
PI 500332	*C. lanatus* var. *citroides*	II	Zambia
PI 512854	*C. lanatus* var. *citroides*	II	Spain
PI 532624	*C. lanatus* var. *citroides*	II	Zimbabwe
PI 532666	*C. lanatus* var. *citroides*	II	Swaziland
PI 220778	*C. colocynthis*	III	Afghanistan
PI 269365	*C. colocynthis*	III	Afghanistan
PI 386016	*C. colocynthis*	III	Iran
PI 386019	*C. colocynthis*	III	Iran
PI 386025	*C. colocynthis*	III	Iran
PI 388770	*C. colocynthis*	III	Morocco
PI 432337	*C. colocynthis*	III	Cyprus

[a]PI designated in Germplasm Resource Information Network (GRIN; www.ars-grin.gov) as *C. lanatus var. lanatus*. However, it contains cpDNA markers present in both *C. lanatus* subspecies (groups I or II).
[b]PI designated in GRIN as *C. lanatus* var. *citroides*. However, it contains cpDNA markers present in both *C. lanatus* subspecies (groups I or II).
[c]PI designated in GRIN as *C. lanatus* var. *lanatus*. However, it contains cpDNA markers unique to *C. lanatus* var. *citroides* (group II).

enzymes: *DraI*, *EcoRI*, *EcoRV*, *HindIII*, or *XbaI*, according to manufacturer's (New England Biolabs, Inc., Beverly, Massachusetts) recommendations. Digestion products were separated through electrophoresis on a 0.9% agarose gel (Agarose-1000; Invitrogene Life Technology, Carlsbad, California). The gels were stained with 0.5 μg/mL ethidium-bromide solution for 25 min and destained for 45 min in distilled water. DNA fragments were visualized under UV light and photographed using a still video system (Gel Doc 2000, Bio-Rad, Hercules, California). DNA was transferred to a nylon membrane (Biodyne B/Plus Membrane, 0.45 μm, Gelman Laboratory, Ann Arbor, Michigan) following Sambrook et al. (1989). CpDNA fragments: P1, P3, P4, P6, P8, P10, P14, and S8 (Systma and Gottlieb 1986), and mtDNA cosmids: cos6, cos7, cos13, cos15, cos18, and cos19 (Hiesel et al. 1987) and clones [*atp*9 (Dewey et al. 1985), *cox*1 (Isaac et al. 1985), and *cob* (Dawson et al. 1984)] were random primed and hybridized to nylon membranes following Sambrook et al. (1989).

Data analysis

A pairwise similarity matrix was generated using the Nei–Li similarity index (Nei and Li 1979) as follows: Similarity $= 2\ N_{ab}\ /\ (N_a + N_b)$, where N_{ab} is the number of RFLP fragments (cpDNA + mtDNA) shared by two genotypes (a and b), and $N_a$and N_b are the total number of RFLP fragments analyzed in each genotype. A dendrogram was constructed based on the similarity matrix data by applying the UPGMA clustering

Table 2. Polymorphic markers in chloroplast and mitochondrial genomes differentiating among the watermelon cultivars (CV), *Citrullus lanatus* var. *lanatus* PIs (CLL), *C. lanatus* var. *citroides* PIs (CLC), and *C. colocyntis* PIs (CC).

Probe	Enzyme	Fragment[a]	CV	CLL	CLC	CC
P1[b]	EcoRI	4.5	0[d]	1[e]	1	1
P1	EcoRI	4.1	1	0	0	0
P1	EcoRI	3.5	0	1	1	1
P1	EcoRI	3.2	1	0	0	0
P1	Hind III	1.6	0	0	0	1(P)[f]
P1	Hind III	1.4	1	1	1	0
P1	Hind III	1.3	0	0	0	1(P)
P1	Hind III	1.2	1	1	1	0
P6	XbaI	11.0	0	0	0	1
P6	XbaI	10.0	0	0	1	0
P6	XbaI	9.0	1	1	0	0
P6	XbaI	4.4	1	1	0	0
P6	XbaI	3.9	0	0	1(P)	1
P6	XbaI	3.7	1	1	0	0
P6	XbaI	2.6	1	1	1	0
P8	DraI	14	1	1	0	0
P8	DraI	1.8	0	0	1	1
S8	EcoRI	4.6	0	0	1(P)	1
S8	EcoRI	4.4	0	1(P)	1(P)	0
S8	EcoRI	4.2	1	1	0	0
cob[c]	EcoRI	1.8	0	0	1(P)	1
cob	EcoRI	1.7	1	1	1(P)	0
atp9	DraI	5.9	0	0	1	1(P)
atp9	DraI	5.7	1(P)	1	0	0
atp9	DraI	5.4	0	0	0	1(P)
atp9	DraI	5.2	1(P)	0	0	0
Cos7	EcoRI	1.6	1	1	1	0
Cos13	HindIII	4.1	0	0	0	1
Cos13	HindIII	3.2	1	1	1	0
Cos13	HindIII	2.7	0	0	0	1

[a]Fragment size in Kilo-base (Kb).
[b]Chloroplast DNA probe.
[c]Mitochondrial DNA probe.
[d]Absent marker.
[e]Marker is present.
[f]Indicates that marker is polymorphic among PIs of the same phenetic group.

algorithm using the Numerical Taxonomic and Multi-Variant Analysis System for PC (NTSYS-PC version 2) (Rohlf 1993).

Results and discussion

Chloroplast genome

The cpDNA probes used in this study cover diverse regions of chloroplast genome of higher plants (Sytsma and Gottlieb 1986) but could only detect a few polymorphisms among chloroplast genomes of *Citrullus* species and subspecies (Tables 1 and 2; Figure 1). The 12 chloroplast-probe/enzyme combinations produced 37 markers. Of these, 17 markers were common to all cultivars and PIs. Common markers were produced by: P1/*HindIII* (14 Kb), P1/*EcoRI* (0.7 and 1.4 Kb), P3/*HindIII* (4.7, 5.1 and 8 Kb), P4/ *EcoRI* (0.8, 1.3, 4.1, and 4.9 Kb), P6/*XbaI* (3.0 Kb), P8/*HindIII* (2.3 and 2.6 Kb), P8/*DraI* (12 Kb), P10/*EcoRI* (5.3 Kb), P14/ *EcoRI* (1.8 and 2.3 Kb). Twenty markers were polymorphic among the major *Citrullus* groups (Table 2). The probe/enzyme combination P6 / *XbaI* produced the highest number of polymorphic markers (Table 2; Figure 1). The cpDNA markers differentiated the cultivars and PIs into three

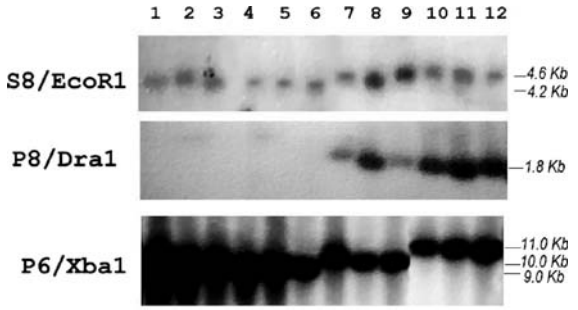

Figure 1. Autoradiograms showing hybridization of ^{32}P-labelled S8, P8 or P6 fragment to total DNA digested with restriction enzymes *EcoRI*, *DraI* or *Xba1* (respectively). Autoradiograms demonstrating polymorphisms among the chloroplast genomes of watermelon cultivars ('Allsweet', 'Charleston Gray' and 'Black Diamond'; lanes 1–3; Group I), *Citrullus lanatus* var. *lanatus* accessions (PI 162667, PI 169289, and PI 185635; lanes 4–6; Group I), *C. lanatus* var. *citroides* (PI 271778, PI 296341, and PI 500332; lanes 7–9; Group II), and *C. colocynthis* (PI 386016, PI 388770, and PI 432337; lanes 10–12; Group III in Figure 3).

distinct groups: *C. lanatus* var. *lanatus* PIs and watermelon cultivars (group I), *C. lanatus* var. *citroides* PIs (group II), and *C. colocynthis* PIs (group III) (Tables 1 and 2; Figures 1 and 3). As expected, the chloroplast genome of watermelon cultivars is closely related to that of *C. lanatus* var. *lanatus*. Two markers produced by P1/*EcoRI* appeared to be unique to cpDNA of the cultivars, distinguishing them from the *C. lanatus* var. *lanatus*PIs (Table 2). The chloroplast genome of *C. colocynthis* is more similar to that of *C. lanatus* var. *citroides* than to that of *C. lanatus* var. *lanatus* (Table 2; Figures 1 and 3). These data complement the analyses based on isozyme (Navot and Zamir, 1987), SSR (Jarret et al. 1997), and RAPD markers (Levi et al. 2001a) which indicated that *C. colocynthis* has comparable genetic distance from both *C. lanatuas* var. *lanatus* and *C. lanatus* var. *citroides*.

Few cpDNA markers were polymorphic within each of the *Citrullus* groups (Table 2; Figure 1). Among them was a 4.2 Kb marker (S8/*EcoRI)* common to cultivars and *C. lanatus* var. *lanatus* accessions, except for PI 270306 (not shown) which has a 4.4 Kb marker in common with *C. lanatus* var. *citroides* PI 189225, PI 244019, PI 179881, PI 248774, PI 271778 and PI 296341 (the marker as shown for the latter two PIs in Figure 1). The rest of the *C. lanatus* var. *citroides* PIs (PI 500332, PI 512854, PI 532624, and PI 532666) have a 4.6 Kb marker (S8/*EcoRI*) common to all *C. colocynthis* PIs (Figure 1).

PI 189225 and PI 271778 were reported as sources of resistance to gummy stem blight (Sowell and Pointer 1962; Sowell 1975). The latter PI is designated as *C. lanatus* var. *lanatus* (GRIN; www.ars-grin.gov). However, based on cpDNA analysis, it falls into the *C. lanatus* var. *citroides* group together with PI 189225 (Table 1; Figure 3). Similarly, PI 270306 and PI 179881 are classified as *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*, respectively (GRIN; www.ars-grin.gov). However, their cpDNA contains markers of both *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* groups, indicating that they might be genetically intermediate between these two groups. The probe/enzyme combination P8/*DraI* produced a 1.8 Kb marker common to all *C. lanatus* var. *citroides* and *C. colocynthis* PIs but missing in all *C. lanatus* var. *lanatus* PIs and cultivars (Figure 1). The same probe/enzyme combination also produced a 14 Kb marker (figure not shown) unique to the cultivars and *C. lanatus* var. *lanatus* PIs (Table 2). The cpDNA pattern shared by all five cultivars (Table 2) indicates that they are derived from common ancestors as suggested in a previous study with RAPD markers (Levi et al. 2001a). It may also reflect a traditional breeding practice, in which watermelon varieties are used as female parents while wild watermelon accessions are used as male parents (pollinators). This practice is designed to eliminate any undetected seed borne viruses or diseases that may exist in wild watermelon accessions, and to take advantage of the soft rind and flesh of cultivated watermelons that are more amenable for seed extraction than those of wild types used in watermelon breeding programs (Gary Elmstrom, personal communication). As a result of maternal transmission of organelles in crosses among *Citrullus* PIs and watermelon cultivars (Havey et al. 1998; Levi unpublished data), the same chloroplast genome may have been maintained in cultivated watermelon during many years of cultivation.

Recently, Dane et al. (2004) examined polymorphisms among chloroplast genomes of *Citrullus* species using the PCR-RFLP procedure (Dumolin-Lapegue et al. 1997). In that study, DNA primer pairs specific to DNA sequences of chloroplast genome from various plant species were used for PCR amplification with watermelon

DNA. Amplified DNA fragments were then digested with restriction enzymes and polymorphism was examined by gel electrophoresis. They identified a few cpDNA haplotypes, each unique to one of the *Citrullus* species or subspecies, and, as in this study, indicated that the cpDNA markers were mostly polymorphic among chloroplast genomes of the major *Citrullus* groups and less polymorphic within them. Dane et al. (2004) also examined the *C. colocynthis* PI 220778 and PI 269365 and the *C. lanatus var. citroides* PI 189225, PI 296341 and PI 512854 (Table 2). These PIs were assembled in their respective groups, as in this study (Figure 3). The cpDNA probe/restriction enzyme combinations used in this study and the cpDNA sequences examined by Dane et al. (2004) identified few polymorphic regions that will be useful in future studies designed to characterize changes that occurred in chloroplast genome along the evolution of *Citrullus* and the development of cultivated watermelon.

Mitochondrial genome

Although mitochondrial genomes are polymorphic in most plant species (Unseld et al. 1997; Bock 2001), only a few polymorphic markers (Table 2) could be detected in this study. Most markers produced by the 12 mitochondrial-probe/enzyme combinations were common to all watermelon cultivars and *Citrullus* accessions. Twenty-one common markers were produced by atp9/*HindIII* (2.5 Kb), Cos6/*HindIII* (5 and 4 Kb), Cos7/*EcoRI* (4 and 3.4 Kb), Cos15/*EcoRV* (5.3 Kb), Cos18/*DraI* (5 and >20 Kb), Cos19/*XbaI* (5.2 Kb), coxI/*EcoRI* (3 Kb), coxI/*HindIII* (1.5 and 3.5 Kb), cob/*EcoRI* (1.3 Kb), cob/*HindIII* (3.3 Kb), Cos13/*HindIII* (1.0, 1.2, 1.4, 1,8, and 2.4 Kb), Cos18/*DraI* (6.0, and >14 Kb).

Ten polymorphic mtDNA markers (Table 2) differentiated the cultivars and PIs into the same three major groups shown by cpDNA analysis (Figure 3). Distinct differences exist between the mitochondrial genomes of all *C. lanatus* groups and the *C. colocynthis* group as shown with markers produced by Cos13/*HindIII* (Figure 2), and Cos7/*EcoRI* (Table 2). Still, as for cpDNA, the mtDNA of *C. colocynthis* more closely resembled that of *C. lanatus* var. *citroides* than that of *C. lanatus* var. *lanatus* (as shown with cob/*EcoRI* and with atp9/*DraI* in Figure 2). Polymorphism among accessions within the same phenetic group occurred with atp9/*DraI* revealing a 5.2 Kb marker (common to cultivars 'Allsweet', 'Charleston Gray', 'Dixie-Queen', and 'New Hampshire Midget'), a 5.7 Kb marker (common to 'Black Diamond' and all *C. lanatus* var. *lanatus* accessions), and a 5.9 Kb marker (common to all *C. lanatus* var. *citroides* and *C. colocynthis* PIs, except for PI 387770 which had a 5.4 Kb marker) (Figure 2).

Sequencing data for *Arabidopsis thaliana* genomes revealed that its chloroplast and mitochondrial genome sizes are 155 Kb (Sato et al. 1999) and 367 Kb (Unseld et al. 1997), respectively. These genomes contain 128 genes (87 protein-coding genes, 4 ribosomal genes, and 37 tRNA genes) and 59 genes (29 protein-coding genes, 5 rRNA genes and 25 tRNA genes), respectively. The chloroplast and mitochondrial genomes of higher plants are small in comparison with the nuclear genome (125-Mb and 25,498 genes in *Arabidopsis thaliana*; Nature 2000). Chloroplast genomes of cucurbits including cucumber *Cucumis sativus* L. (Palmer 1982), squash *Cucurbita pepo* L. (Lim et al. 1990), and melon *Cucumis melo* L. (Perl-Treves and Galun 1985) have similar size (150–155 kb) and structure. However, significant differences exist in the mitochondrial genomes of these cucurbit species (Ward

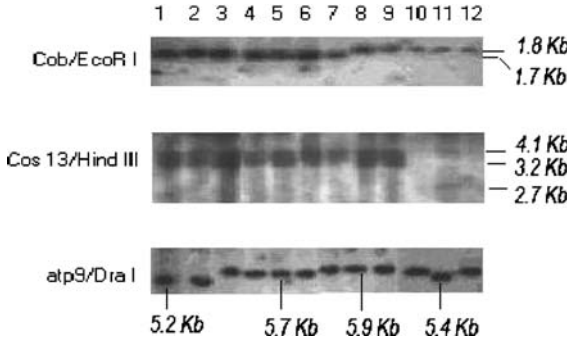

Figure 2. Autoradiograms showing hybridization of ^{32} P-labeled cob, Cos13 or atp9 fragment to total DNA digested with restriction enzymes *EcoRI*, *HindIII* or *DraI* (respectively). Cultivars and PIs are in the same order as shown in Figure 1. The autoradiograms demonstrate polymorphisms among mitochondrial genomes of watermelon cultivars (lanes 1–3; Group I), *Citrullus lanatus* var. *lanatus* (lanes 4–6; Group I), *C. lanatus* var. *citroides* (lanes 7–9; Group II), and *C. colocynthis* (lanes 10–12; Group III in Figure 3).

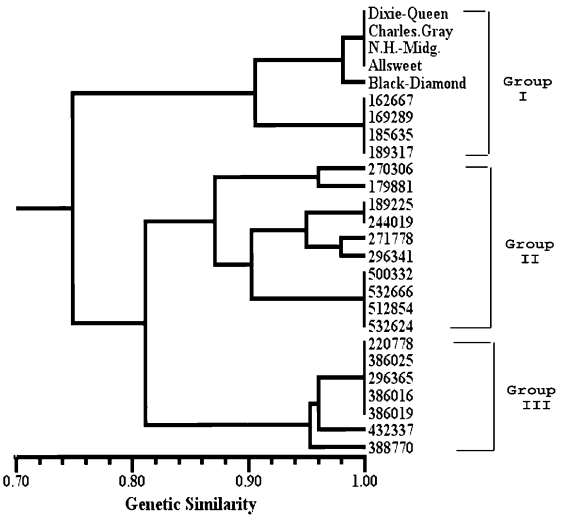

Figure 3. Dendrogram showing phenetic relationships among the three major *Citrullus* PI groups in this study: watermelon cultivars and *C. lanatus* var. *lanatus* PIs (Group I), *C. lanatus* var. *citroides* PIs (group II), and *C. colocynthis* PIs (group III). The dendrogram was produced by UPGMA cluster analysis of similarity matrix based on cpDNA and mtDNA polymorphic markers (Table 2).

et al. 1981; Stern et al. 1986; Lilly et al. 2001; Lilly and Havey 2001). Wolfe et al. (1987) indicated that sequences in nuclear genes of higher plants were the fastest to evolve followed by chloroplast genes, while mitochondrial genes were the slowest to evolve. Conversely, extensive rearrangements have occurred in the structural organization of mitochondrial genomes of higher plants (Laroche et al. 1997; Adams et al. 2002).

Conclusions

Chloroplast and mitochondrial genomes of watermelon are generally conserved, and are likely to evolve at a slower rate than is the nuclear genome, as has been shown for other higher plant species (Wolfe et al. 1987; Laroche et al. 1997). As shown in this study (with PI 179881, PI 270306, and PI 271778) polymorphic cpDNA or mtDNA markers can be useful in complementing nuclear genome data for classifying valuable *Citrullus* PIs collected from various geographical regions (Dane et al. 2004). Higher similarity exists between chloroplast and mitochondrial genomes of *C. colocynthis* and *C. lanatus* var. *citroides* (of *C. lanatus* subsp. *lanatus*) than with these of the cultivated watermelon (of *C. lanatus* subsp. *vulgaris*) which is designated in this study as *C. lanatus* var. *lanatus*. Cultivated watermelon has a narrow genetic base (Levi et al. 2001b). The collection of 1600 *Citrullus* PIs at the USDA, ARS, Plant Genetic Resources Conservation Unit (Griffin, Georgia) (GRIN; www.ars-grin.gov) is potentially a valuable source for enhancing watermelon cultivars (Jarret et al. 1997; Levi et al. 2001a; Dane et al. 2004). Chloroplast and mitochondrial genomes of the wild *Citrullus* types might also be valuable in genetic enhancement of watermelon cultivars, providing that they are compatible with their nuclear genome. This was the case in a study examining interaction between cytoplasmic genomes of the wild grass teosnite and the Maize nuclear genome (Allen et al. 1989). The polymorphic cpDNA and mtDNA markers identified in this study have been useful in our experiments (data not shown) designed to replace the chloroplast and mitochondria of cultivated watermelon with those of *C. colocynthis* (through repeated backcrosses using *C. colocynthis* as the maternal parent in the initial cross, while using watermelon cultivars as pollinators in following backcrosses). Our experiments confirmed maternal inheritance in crosses between watermelon cultivars and *C. colocynthis* PIs as indicated by Havey et al. (1998) for crosses between *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*.

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